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RESEARCH ARTICLE

DEVELOPMENT OF NEW SOLVENT SYSTEMS FOR THE ANALYSIS OF DIAZEPAM FROM BLOOD

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ABSTRACT: Diazepam is a medication of the benzodiazepine family that typically produces a calming effect. It is commonly used to treat a range of conditions including anxiety, alcohol withdrawal syndrome, muscle spasms, seizures, trouble sleeping, and restless legs syndrome. Diazepam was analyzed by using different instrumentation techniques such as HPLC, GLC, LC-MS, GC-MS etc. An attempt has been made to develop a new method for analysis of Diazepam in Biological sample such as blood using High Performance Thin Layer Chromatography (HPTLC) Plate. Diazepam was extracted from blood using liquid-liquid extraction method and analyzed by using High Performance Thin Layer Chromatography Plate. For Chromatographic separation, various binary and tertiary solvent systems were used as mobile phase. Fifteen different solvent systems with different volumetric ratios were used in separation and identification of Diazepam. Developed plates were viewed under UV light followed by spray of chromogenic reagents which successfully increased the sensitivity without dispensing the simplicity of the method. The method developed is a simple, rapid, expensive, non–destructive and reproducible which can be performed in any laboratory easily.

KEYWORDS: Diazepam, Benzodiazepine, Solvent Systems, TLC, Rf, Spraying Reagent etc.

INTRODUCTION:

Diazepam was invented by Dr. Leo Steinbach Hoffmann-La Roche at the company's Nutley, New Jersey, facility following chlordiazepoxide (Librium), which was approved for use in 1960.[1] It is commonly used to treat a range of conditions including anxiety, alcohol withdrawal syndrome, muscle spasms, seizures, trouble sleeping, and restless legs syndrome. It may also be used to cause memory loss during certain medical procedures. It can be taken by mouth, inserted into the rectum, injected into muscle or vein. When given into a vein, effects begin in one to five minutes and last up to an hour. By mouth, effects may take 40 minutes to begin. Common side effects include sleepiness and trouble with coordination. Serious side effects are rare. Decreased breathing, and an increased risk of seizures if used too frequently. Long term use can result in tolerance, dependence,

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and withdrawal symptoms on dose reduction. Abrupt stopping after long-term use can be potentially dangerous. After stopping, cognitive problems may persist for six months or longer. It is not recommended during pregnancy or breastfeeding. Its mechanism of action is by increasing the effect of the neurotransmitter *gamma*-Amino butyric acid (GABA). Diazepam was first synthesized by Leo Steinbach, and was first manufactured by Hoffmann-La Roche. Routinely, Diazepam was analyses by using Thin Layer Chromatography (TLC)[2-4] High Performance Liquid Chromatography (HPLC)[5-7], UV-Visible Spectrophotometer [8], Gas Liquid Chromatography (GLC)[9-11], Gas Chromatography-Mass Spectroscopy (GC-MS) [12] and Liquid Chromatography-Mass Spectroscopy (LC-MS) [13]. It has been one of the most frequently prescribed medications in the world since its launch in 1963[14]. In the United States it was the highest selling medication between 1968 and 1982, selling more than two billion tablets in 1978 alone [15]. In 1985 the patent ended, and there are now more than 500 brands available on the market. Diazepam is on the World Health Organization's List of Essential Medicines; the most effective and safe medicines needed in a health system [16]. The wholesale cost in the developing world is about 0.01 USD per dose as of 2014[17]. In the United States it is about 0.40 USD per dose [18]. Diazepam can be administered orally, intravenously (must be diluted, as it is painful and damaging to veins) intramuscularly (IM), or as a suppository [19].

MATERIAL AND REAGENTS:

Chemicals

Methanol, Ammonia, Chloroform, Acetic Acid, Toluene, Butanol, Benzene, Diethyl ether, Ethanol, Conc. Hydrochloric acid, Sulphuric acid, anhydrous Sodium sulphate,n-Hexane, Carbon Tetrachloride, Xylene, N, N-Diethylformamide, Sodium tungstate dihydrate and Amyl alcohol, Cyclohexane, Acetonitrile, Paraffin Paper, Iodine etc from Merck India was used.

Glassware

Beaker, conical flask, chromatographic chamber, measuring cylinder, separating funnel, volumetric flask,TLC spreyer from Borosil India were used. Microcapillaries from Top Tech Lab Equipments Pvt. Ltd. was used.

Ultrapure water

Ultrapure water from Rions India was used throughout.

High Performance Thin layer chromatography (HPTLC) plates

HPTLC Silicagel 60 F_{254} aluminium sheets from Merck Germany were used.

Miscellaneous

UV chamber, oven, Scale, Pencil, Digital weighing balance etc from different manufacturer were used.

- **Preparation of Standard solution**

1000 ppm solution of Diazepam was prepared by dissolving 0.1 gm of Diazepam in 100 ml of methanol.

- **3.Extraction of Diazepam Poison from Blood**

5ml of blood sample was taken in a beaker, pinch of sodium tungstate and 1-2 ml of H_2SO_4 was added to it and heated at 60^0C for 2-3 minutes. Contents are filtered through filter paper and washed with 0.1N sulphuric acid. The filtrate was made alkaline with ammonia and mixed with ether:chloroform (3:1) and shaken well. Organic layer was collected. The collected organic layer was dried. The dried residue is mixed with few drops of methanol which was used for spotting on TLC plate.

- **Spotting of sample and standard on HPTLC plates**

Diazepam extracted from blood was loaded on the HPTLC plates along with the standard.

- **Development of the TLC Plates**

Spotted plates were developed in different solvent systems taken in different ratios (Table 1). After

developing, the TLC plates were taken out from the solvent chamber and air-dried.

- **Visualization of TLC Plates**

The dried and developed TLC plates were first observed under UV and then kept in iodine chamber for visualization.

RESULT AND DISCUSSION:

The dried and developed TLC plates were first observed under UV and then kept in iodine chamber for visualization. There were 15 different solvent systems (Table 1) with different volumetric ratios were used. The R_f value was also varying with different volumetric ratios. The R_f value of Diazepam extracted from blood under experimental conditions was found nearly equal to standard . The response of separation of diazepam in all the 15 solvent system were observed along R_f value and presented in the Table 1.

Table 1. Solvent system with ratio and R_f values

S.No.	Solvent System	Ratios	R_F of Standard	R_F of Sample
1.	Diethyl ether: acetone	5:5	0.97	0.97
		6:4	0.98	0.98
		7:3	0.98	0.98
		8:2	0.92	0.92
		9:1	0.97	0.97
		4:6	0.96	0.96
		3:7	0.97	0.96
		2:8	0.98	0.97
		1:9	0.98	0.98
2.	Chloroform: methanol	5:5	0.97	0.92
		6:4	0.86	0.85
		7:3	0.84	0.83
		8:2	0.86	0.85
		9:1	0.87	0.85
		4:6	0.84	0.82
		3:7	0.89	0.85
		2:8	0.76	0.74
		9:1	0.88	0.86
3.	Chloroform: Toluene	5:5	0.90	0.88
		6:4	0.93	0.92
		7:3	0.78	0.66
		8:2	0.79	0.77
		9:1	0.88	0.85
		4:6	0.92	0.91
		3:7	0.89	0.85
		2:8	0.92	0.92
		1:9	0.82	0.82
4.	Diethyl ether: Methanol	5:5	0.87	0.84
		6:4	0.89	0.87
		7:3	0.88	0.87
		8:2	0.89	0.86
		9:1	0.86	0.82
		4:6	0.88	0.88
		3:7	0.87	0.87
		2:8	0.89	0.85
		1:9	0.88	0.85
5.	Ethanol: Methanol	5:5	0.73	0.72
		6:4	0.72	0.71
		7:3	0.78	0.74
		8:2	0.72	0.71
		9:1	0.80	0.78
		4:6	0.77	0.76
		3:7	0.87	0.86
		2:8	0.91	0.85
		1:9	0.88	0.85
6.	Diethyl ether: Carbon tetrachloride	5:5	0.25	0.23
		6:4	0.30	0.30
		7:3	0.39	0.36
		8:2	0.39	0.35
		9:1	0.52	0.49
		4:6	0.28	0.28
		3:7	0.29	0.27
		2:8	0.31	0.26
		1:9	0.31	0.28
7.	Acetic acid: Acitonitrile	5:5	0.79	0.78
		6:4	0.91	0.91
		7:3	0.83	0.80
		8:2	0.80	0.78
		9:1	0.77	0.69
		4:6	0.82	0.78
		3:7	0.89	0.82
		2:8	0.83	0.74
		1:9	0.76	0.72
8.	Xylene: Diethyl ether	5:5	0.24	0.24
		6:4	034	0.28
		7:3	0.22	0.22
		8:2	0.17	0.17
		9:1	0.80	0.60
		4:6	0.32	0.31
		3:7	0.33	0.33

		2:8	0.41	0.38
		1:9	0.39	0.36
9.	Dimethyl formamide: Toluene	5:5	0.86	0.84
		6:4	0.86	0.82
		7:3	0.84	0.80
		8:2	0.89	0.86
		9:1	0.87	0.84
		4:6	0.88	0.85
		3:7	0.83	0.82
		2:8	0.89	0.88
		1:9	0.91	0.89
10	Acetic acid: Butanol	5:5	0.83	0.78
		6:4	0.83	0.83
		7:3	0.79	0.74
		8:2	0.83	0.80
		9:1	0.79	0.75
		4:6	0.87	0.87
		3:7	0.87	0.87
		2:8	0.84	0.83
		1:9	0.81	0.81
11	Acetic acid: Carbon tetra chloride	5:5	0.75	0.72
		6:4	0.73	0.71
		7:3	0.76	0.76
		8:2	0.79	0.76
		9:1	0.70	0.66
		4:6	0.71	0.68
		3:7	0.68	0.62
		2:8	0.55	0.48
		1:9	0.75	0.73
12.	Acetic acid: Toluene	5:5	0.70	0.67
		6:4	0.89	0.85
		7:3	0.91	0.89
		8:2	0.88	0.86
		9:1	0.95	0.92
		4:6	0.75	0.73
		3:7	0.75	0.70
		2:8	0.52	0.47
		1:9	0.27	0.23
13.	Amyl alcohol: cyclohexane	5:5	0.99	0.98
		6:4	0.95	0.93
		7:3	0.98	0.98
		8:2	0.55	0.51
		9:1	0.51	0.47
		4:6	0.98	0.97
		3:7	0.74	0.70
		2:8	0.82	0.78
		1:9	0.86	0.80
14.	Amyl alcohol: Chloroform	5:5	0.89	0.86
		6:4	0.80.	0.76
		7:3	0.86	0.81
		8:2	0.89	0.79
		9:1	0.76	0.71
		4:6	0.78	0.74
		3:7	0.88	0.82
		2:8	0.91	0.87
		1:9	0.95	0.91
15.	Cyclohexane: acetone	5:5	0.97	0.92
		6:4	0.88	0.83
		7:3	0.82	0.76
		8:2	0.92	0.85
		9:1	0.78	0.71
		4:6	0.88	0.88
		3:7	0.86	0.82
		2:8	0.79	0.71
		1:9	0.91	0.89

CONCLUSION:

Method developed for the detection of diazepam in blood is very simple, easy to perform in any laboratory with very less time and reproducible result. This method can be utilized by Forensic Scientist/Chemist of different Forensic Science Laboratory.

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